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THE UNIVERSITY OF ALBERTA

ALKALOIDS OF THE RUTACEAE

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

FACULTY OF PHARMACY

DEPARTMENT OF CHEMISTRY

EDMONTON, ALBERTA

19th November 1962

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Alkaloids of the Rutaceae" submitted by Harold E. McDonald, B.Sc., in partial fulfilment of the requirements for the degree of Master of Science.

Supervisor

19th November 1962

ABSTRACT

In a survey of the alkaloids previously reported in the African Rutaceae it was observed that those compounds could be divided into four groups on the basis of their properties and especially their paper chromatographic characteristics. Several techniques for the separation of the alkaloids, including partition and adsorption column chromatography and thin layer chromatography on silica gel, were investigated.

Examination of the alkaloids of the stem bark of Fagara melanacantha (Planch.) Eng. resulted in the isolation of four alkaloids. Of these, melanacanthine and melanacanthinine had previously been isolated from this species. The other two alkaloids were skimmianine which is a common constituent of the Rutaceae, and Base 4 which was shown to be 2,3-dimethoxy-4-hydroxy-10-methyl-acridone. The latter is the first acridone alkaloid to be isolated from either of the genera Fagara or Xanthoxylum.

Xanthofagarine, the major alkaloid of the root bark of F. macrophylla (Oliver) Eng., was isolated and shown to be 2',3'-methylenedioxy-6,7-dimethoxy-10-methyl-1,2-benzophenanthridine. In addition two other bases, and fagaramide were obtained.

ACKNOWLEDGMENTS

The author gratefully acknowledges the valuable assistance and encouragement offered by Dr. K. H. Palmer and Dr. W. A. Ayer during the course of this investigation. He also wishes to thank his colleagues in the laboratory for technical assistance and interest in the project.

The writer also acknowledges the financial assistance given by the National Research Council of Canada, and the material assistance of the Government of Ghana, and especially of the Conservator of Forests, in the supply of the necessary plant material.

Finally the author wishes to express appreciation to his wife for her encouragement and assistance in the preparation of the manuscript, and for the typing of this thesis.

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INTRODUCTION

Plants of the Natural Order Rutaceae are widely distributed throughout the tropical and sub-tropical regions of the world. Alkaloids have been reported in several genera of this family, though the species of the best known genus - Citrus - rarely contain alkaloids.

The work to be described later is concerned with two West African species of Fagara: F. melanacantha (Planchon) Engler and F. macrophylla (Oliver) Engler. Members of the genus Fagara, of which more than two hundred species are known, usually occur as trees or shrubs whose stems, branches and twigs usually bear thorns.

It is of interest, by way of introduction, to study not only the work previously done on the alkaloids of the genus Fagara, but also to obtain an insight into the types of alkaloids which have been isolated from the various genera of the Rutaceae.

The family Rutaceae is particularly well represented in Africa and Australia and in recent years a considerable volume of work has been done on alkaloids of the Australian Rutaceae. The genera which have been most extensively studied are: Acronychia, Evodia, Flindersia, Lunasia and Xanthoxylum.

The furoquinoline alkaloid, acronycidine (5,7,8,-trimethoxy-dictamnine) was isolated from the bark of Acronychia baueri Schott (1, 2) and also from the bark of Melicope fareana F. Muell. (3). The leaves of A. baueri (4) yielded eight alkaloids, of which six were already known

from other sources: melicopicine, melicopidine and melicopine from M. fareana (3, 5), acronycidine from M. fareana (3) and the bark of A. baueri (1), skimmianine from Skimmia repens Nakai (6) and many Rutaceous plants, and kokusaginine from Orixa japonica Thunb. (7). The two new alkaloids were: 2,4-dimethoxy-10-methyl-acridone and acronidine (4), a furoquinoline derivative.

Evodia xanthoxyloides F. Muell. (8) yielded a total of ten alkaloids from the bark, of which nine have been characterised. Of these melicopidine was already known. The others were the acridone alkaloids evoxanthidine, xanthevodine, xanthoxoline and 4-hydroxy-2,3-dimethyoxy-10-methyl-acridone (9), and three furoquinoline derivatives, evoxine (10), evodine (11) and evoxoidine (10).
E. littoralis Endler (12) yielded dictamnine, previously isolated from Dictamnus albus L. (13), and many other Rutaceae, kokusaginine and the new furoquinoline alkaloid evolitrine. The known alkaloids evoxanthine, melicopidine and kokusaginine, a new acridone alkaloid 2,3,4-trimethoxy-10-methyl-acridone and a new furoquinoline, evolatine, were obtained from the bark of E. alata F. Muell. (14).

Early workers on Flindersia australis R. Br. (15), reported the isolation of flindersine in 2% yield. An alkaloid differing only slightly from the description of flindersine was later isolated and shown to have a 2,3-pyrano-quinoline skeleton (16). F. acuminata C. T. White (17) was found to contain dictamnine and maculine. F. maculosa Lindl. and F. dissosperma F. Muell. are, in composition, chemically

very similar (18), but the alkaloid content was found to vary greatly from sample to sample. The former gave maculine (19), maculosidine (20), maculosine (20), kokusaginine, skimmianine and dictamnine; all furoquinoline derivatives, and a new alkaloid, flindersiamine. The latter (18) gave skimmianine, dictamnine and flindersiamine.

The species Medicosma cunninghamii Hook. f. which is the only member of its genus, yielded medicosmine (21), which is a furoquinoline alkaloid with a pyran ring fused in either the 5,6- or 6,7- positions.

In the genus Xanthoxylum, X. brachyacanthum F. Muell. and X. veneficium F. M. Bail., are probably identical (22). The bark of both species contained β-homochelidonine (α-allocryptopine), l-α-canadine and chelerythrine, which are benzo-phenanthridine alkaloids, and isocorydine methiodide. The leaves were found to contain only β-homochelidonine. X. suberosum C. T. White was reported to contain canthin-6-one (22), which was first identified as a constituent of Pentaceras australis Hook. f. (23).

The furoquinoline alkaloid lunacrine was isolated from Lunasia costulata Miq. in 1900 (24), and the related quinoline derivative lunacridine was reported in 1943 (25). At lease fifteen bases have been isolated from L. amara Blanco. of Phillipine origin. In addition to the well known alkaloids skimmianine and kokusaginine, the furoquinoline group was well represented by: lunacrine (26, 27), lunine (26), hydroxylunacrine and hydroxylunine (28). Lunacridine (27), hydroxylunacridine (29) and hydroxylunidine (28) are thought

to be artefacts formed from the lunacrinium ion under the alkaline conditions which were used in extraction. The remainder of the alkaloids obtained were substituted quinolines and quinolones. Lunacrinol is a pyranoquinol-4-one (30) biogenetically related to the 2-isopropyl furoquinolines.

Dutch workers (30, 31) reported the isolation of six alkaloids from the same species, some of which have been correlated with those described above. An interesting feature of this work was the isolation of a 3,4-(2'-isopropyl 2'3'-dihydrofuro)2-quinolone isomeric with lunacrine and probably formed from lunacridine by the action of acid.

In Australia, the species Lunasia quercifolia K. Schum. has been found to contain the alkaloids lunacrine, lunine and 7-methoxy-methyl-2-phenyl-4-quinolone (32, 33). A mixture of salts derived from the lunacrinium ion was also obtained.

A Japanese species, Phellodendron amurense Rupr., was found to contain berberine and palmitine (34). Further examination of the bark of P. amurense showed the presence, in addition to the berberine type alkaloids mentioned above, the quaternary bases, guanidine, phellodendrine and magnoflorine (35). The last named had previously been isolated from Magnolia grandiflora L. (36). Phellodendrine has since been found to be identical to L-(-)-N-methyl-coreximine (37, 38). P. amurense var. sacchaliense yielded, in addition to most of the alkaloids mentioned above, the berberine alkaloid, jatrorrhizine (39). Also in Japan, dictamnine, skimmianine and magnoflorine have been obtained from the roots of Xanthoxylum planispium (40).

X. nitidum D. C., and X. avicennae (Lam.) D. C., which are native to Hong Kong, have yielded, the former species oxynitidine and salts of the unstable quaternary base nitidine (41), and the latter species the related alkaloids avicine and oxyavicine (42); all having the benzophenanthridine skeleton (43, 44). Fagara semiarticulata, which is native to Hawaii, was also found to contain benzophenanthridine alkaloids, chelerythrine and dihydro-chelerythrine having been isolated (45). Recently, another Hong Kong species, Ruta graveolens L., has been found to contain kokusaginine, skimmianine and a new alkaloid graveoline (46), which has been shown to be 1-methyl-2-(3,4-methylenedioxy-phenyl)-4-quinolone (46, 47).

The Indian species Xanthoxylum rhetsa D. C., which is identical to X. budrunga D. C., yielded four alkaloids (48): the indoloquinazoline alkaloids rhetsine and rhetsinine (hydroxyrhetsine), and rhetine and chelerythrine. Rhetsine is identical to evodiamine (49).

The early work on the genera Fagara and Xanthoxylum was concerned with those species which are native to South America and Africa. In 1921, in a review of work done on these genera, Goodson (50) recorded that alkaloids had been found in all the species which had been investigated, but that only small quantities occurred except in: F. brachyacantha Engl. (X. brachyacanthum F. Muell.), F. monophylla Lam., F. caroliniana Engl. (X. clavaherculis L.), F. xanthoxyloides Lam. (X. senegalense D. C.) and F. macrophylla Engl. (X. macrophyllum Oliver.). It should be pointed out that classification of these genera is difficult, and often the

same plant may be known under two different names. The nature of the substances isolated in this early work is indicated in Table I.

The most extensively studied species of the genus Fagara is the Argentinian plant, F. coco Engl. (51). In 1933, G. V. Stuckert (52) isolated α-, β-, γ- and δ- fagarines, whilst Merck and Company, working at Stuckert's request, isolated only two alkaloids, fagarine I and fagarine II. α-fagarine and fagarine II were subsequently shown to be α-allocryptopine (51), β-fagarine to be the alkaloid skimmianine (53) and γ-fagarine to be 8-methoxy dictamnine (54). In 1954, two new alkaloids were obtained from this species: fagarine III and fagaridine chloride (identical to N-methyl-isocorydine) (55). The structure of fagarine III, which is similar to α-allocryptopine, has also been determined (56). Two alkaloids of unknown structure have recently been obtained from F. tingoassuiba (A. St. Hil.) Hoehne., which is native to Brazil (57).

From 1947, Paris and Moyse-Mignon, and later Paris and Palmer investigated several species of Fagara from French West Africa. The root of X. senegalense ("Artar Root") had earlier been reported (58, 59) to contain an amorphous, water-insoluble alkaloid, artarine and a blood-red water soluble base. Paris and Moyse-Mignon obtained three alkaloids from F. xanthoxyloides Lam. (X. senegalense) (60) designated A1, A2 and A3, and having the properties indicated in Table II. Alkaloid A1 was found to be skimmianine while alkaloid A3 corresponded to the red alkaloid previously isolated and was

TABLE I

CONSTITUENTS OF FAGARA AND XANTHOXYLUM SPECIES REPORTED BY EARLY WORKERS (50)

Xanthoxylum brachyacanthum	1-α-canadine methochloride
	β-homochelidonine
Fagara monophylla	Two alkaloids: (a) $C_{24}H_{25}NO_6$.
	(b) -
F. caroliniana	Berberine
	Xanthoxylin-S: $C_{14}H_{12}O_4$ or $C_{21}H_{18}O_6$.
X. senegalense	Alkaloid: $C_{21}H_{23}NO_4$.
	Neutral substance: $C_{10}H_{10}O_3$ or $C_{14}H_{14}O_4$.
	Caprinamide
	Xanthotoxin: $C_{12}H_8O_4$.
	Fagarol: $C_{20}H_{18}O_6$.
	Fagaramide: $C_{14}H_{17}NO_3$.
F. macrophylla	Fagaramide
	Lupeol
F. budrunga	Xanthosterin: $C_{23}H_{39}OH$
F. flava	Lactone: $C_{11}H_{10}O_3$.
	Ether-lactone: $C_{14}H_{12}O_3$.
X. fraxineum	Xanthoxylin-N: $C_{15}H_{14}O_4$.

TABLE II

ALKALOIDS OF THE FAGARA AND XANTHOXYLUM SPECIES OF AFRICA (60-67)

Fagara xanthoxyloides	A1:	skimmianine, m.p. 178°C.
	A2:	$C_{23}H_{25}NO_5$, m.p. 200-202°C.
	A3:	fagaridine, $C_{19}H_{24}NO_7$, m.p. 238-240°C.
F. viridis		skimmianine
		artarine, as hydrochloride, m.p. 245°C.
		fagaridine, small quantity only.
F. parvifolia		parvifagarine, $C_{23}H_{21}NO_4$, m.p. 165-166°C.
F. angolensis		angolinine, $C_{24}H_{23}NO_6$, m.p. 270°C.
		angoline, $C_{22}H_{21}NO_4$, m.p. 209°C.
		skimmianine, m.p. 178-179°C.
F. heitzii		α-heitzine, $C_{19}H_{17}NO_4$, m.p. 270°C.
		β-heitzine, $C_{19}H_{17}N_3O_2$, m.p. 262-263°C.
		γ-heitzine, $C_{25}H_{25}NO_5$, m.p. 199-200°C.
		δ-heitzine, $C_{21}H_{19}NO_5$, m.p. 254-255°C.
F. melanacantha		melanacanthinine, $C_{44}H_{46}NO_9$, m.p. 202°C.
		melanacanthine, $C_{23}H_{23}NO_5$, m.p. 209°C.
F. macrophylla	A:	m.p. 209-211°C.
	B:	xanthofagarine, $C_{18}H_{22}NO_8$, m.p. 278°C.
	C:	erythrofagarine, identical to fagaridine.

named fagaridine. Non alkaloidal materials isolated by various workers include: fagarol, pseudo-fagarol, linalool, xanthotoxin and fagaramide; though the last named may have been obtained from an incorrectly classified sample of F. macrophylla, which is very closely related to F. xanthoxyloides.

In a study of F. viridis A. Chev., which had not previously been examined, Paris and Moyse-Mignon (61) found skimmianine and indications of the presence of another alkaloid in the stem bark. In the root bark, skimmianine was not found, however a yellow alkaloid hydrochloride, resembling artarine hydrochloride, and a very small amount of another alkaloid resembling fagaridine were isolated. Fagarol and a substance resembling xanthotoxin were also obtained.

The stem bark of F. parvifolia A. Chev. yielded parvifagarine and several other crystalline alkaloids in small yield. Examination of the root bark gave similar results but only a small quantity of parvifagarine was obtained. A crystalline phytosterol was also isolated (61).

Paris and Palmer re-examined the alkaloids of some of these species, using paper chromatography under standard conditions, and subsequently attacked the problem of separation of the alkaloids by means of column partition chromatography (62). In this work F. angolensis Engl. (63, 64) yielded two new alkaloids, angoline and angolinine, together with traces of skimmianine. Four alkaloids were isolated from the root bark of F. heitzii Aubrev. et Pelleg.:

α-, β-, γ- and δ-heitzine, and the presence of a fifth alkaloid in smaller quantities was shown by paper chromatography (65). Melanacanthine and melanacanthinine were isolated from all parts of F. melanacantha (Planch.) Engl. but the root and stem barks were found to be richest in alkaloids (66).

F. macrophylla Engl., which forms the subject of the major part of this dissertation, was first investigated by Goodson in 1921 (50). The presence of lupeol and fagaramide is reported, but no mention is made of the presence of alkaloids. In 1951, this species was re-investigated by Paris and Moyse-Mignon (67), who obtained fagaramide, and a mixture of phytosterols which was not further studied. Three alkaloids were obtained from the root bark: alkaloid A, xanthofagarine and erythrofagarine. Erythrofagarine was found to be identical to fagaridine, which was previously isolated from F. xanthoxyloides.

It will be seen that certain basic alkaloidal skeletons occur frequently in the Rutaceae. The most commonly occurring type is the furoquinoline group, of which the three alkaloids skimmianine, kokusaginine and dictamnine are repeatedly recurring. Other common types are: acridones, mainly in the Australian Rutaceae; quinolines and quinolones; and 1,2-benzophenanthridines.

Mention must now be made of the pharmacological action of the genera Fagara and Xanthoxylum. The bark of F. macrophylla has long been used in African folk-medicine as a cure for toothache (67). A strong local anaesthetic

action of a concentrated heptane extract of the root bark which has been observed in this laboratory indicated that this treatment may not have been ineffective. X. avicennae is used in Hong Kong in the treatment of sore throat and jaundice (42).

The pharmacological action of the constituents of F. coco has been more systematically examined. Stuckert and Sartori reported that α-fagarine exerts a depressant action on cardiac functions (68). It has been reported to be superior to quinidine for controlling cardiac arrhythmias (69).

Paris and Moyse-Mignon found that injections of root bark extracts of F. macrophylla caused first a decrease and then a slight increase in the blood pressure of anaesthetised dogs (67). The same authors investigated the action of xanthofagarine and found that a subcutaneous injection of 10 grams per kilogram of body weight caused 30% mortality in mice, and that a 0.1 gram per kilogram injection caused bradycardia and hypotension in dogs (67). It was also found to be toxic to fish, death being preceded by a loss of equilibrium (67).

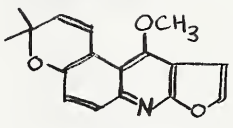
The structures of the alkaloids described in this survey, together with their sources, are given in Table III. A comprehensive list of all members of the Rutaceae which had been reported to contain alkaloids up to the end of 1957 mentions 181 species (70). While the present survey does not cover all of these species, it does include representatives of all the major classes of alkaloids which have been found to date in the Rutaceae.

In view of the paucity of information pertaining to the nature of the alkaloids present in the African Fagara, and of the reported cardioactivity of extracts of F. coco and F. macrophylla, it seemed of interest to investigate further the alkaloids of these species.

TABLE III

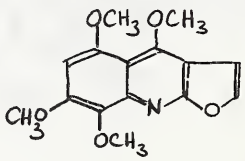
ALKALOIDS OF THE RUTACEAE

ACRONIDINE



Achronychia bauerii, (4)

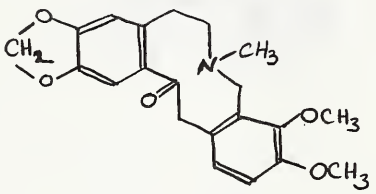
ACRONYCIDINE



A. bauerii, (1, 2)

Melicope fareana, (3)

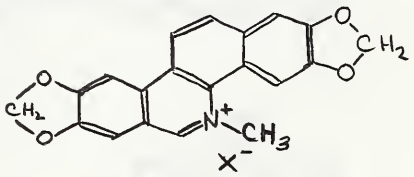
∝-ALLOCRYPTOPINE



Fagara coco, (51)

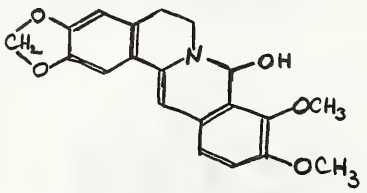
Xanthoxylum brachyacanthum and *X. veneficium*, (22)

AVICINE



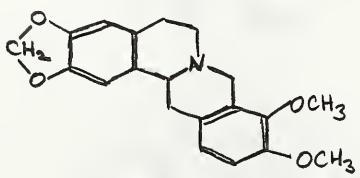
X. avicennae, (42)

BERBERINE



Phellodendron amurense, (34)

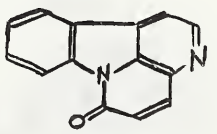
1-∝-CANADINE



X. brachyacanthum, (22)

X. veneficium, (22)

CANTHIN-6-ONE

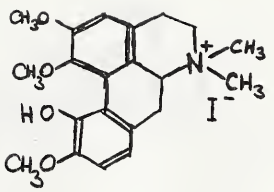


Pentaceras australis, (23)

X. suberosum, (22)

iso-CORYDINE METHIODIDE

F. coco, (55)



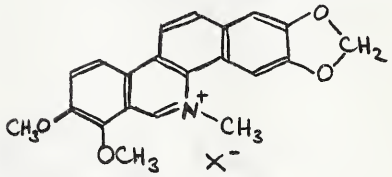
CHELERYTHRINE

X. brachyacanthum, (22)

X. veneficium, (22)

X. rhetsa, (48)

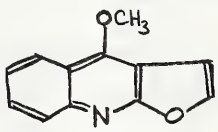
F. semiarticulata, (45)



DICTAMNINE

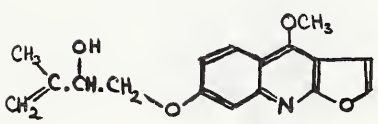
Dictamnus albus, (13)

Many other Rutaceae



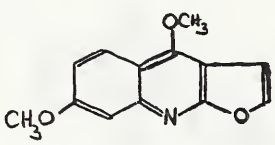
EVODINE

Evodia xanthoxyloides, (11)



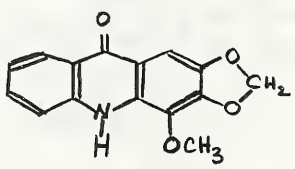
EVOLITRINE

E. littoralis, (12)



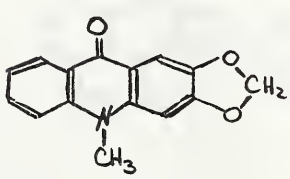
EVOXANTHIDINE

E. xanthoxyloides, (9)



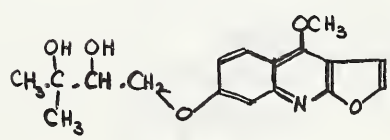
EVOXANTHINE

E. alata, (14)



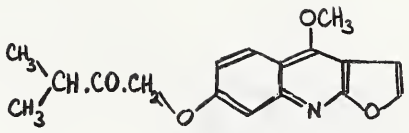
EVOXINE

E. xanthoxyloides, (10)



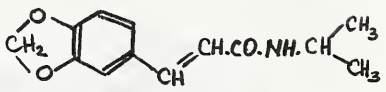
EVOXOIDINE

E. xanthoxyloides, (10)



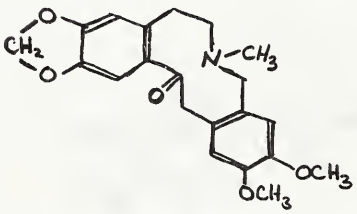
FAGARAMIDE

F. macrophylla, (50)



FAGARINE III

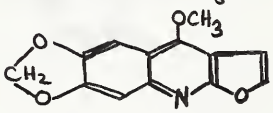
F. coco, (55)



FLINDERSIAMINE

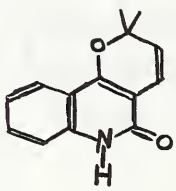
Flindersia dissosperma, (18)

Fl. maculosa, (18)



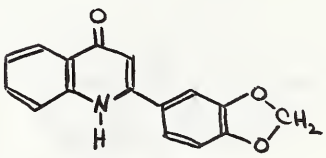
FLINDERSINE

Fl. australis, (15, 16)



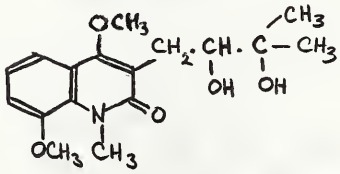
GRAVEOLINE

Ruta graveolens, (46)



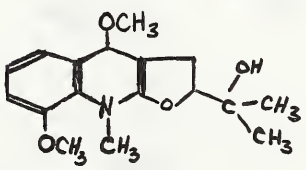
HYDROXYLUNACRIDINE

Lunasia amara, (29)



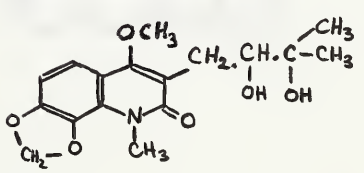
HYDROXYLUNACRINE

L. amara, (28)

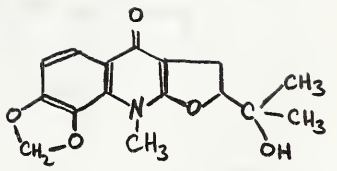


HYDROXYLUNIDINE

L. amara, (28)

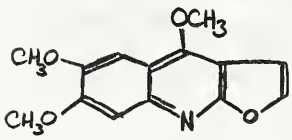


HYDROXYLUNINE



L. amara, (28)

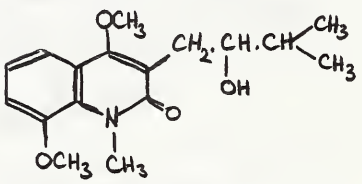
KOKUSAGININE



Orixa japonica, (27)

Many other Rutaceae

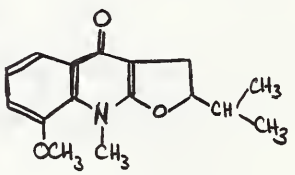
LUNACRIDINE



L. amara, (27)

L. costulata, (25)

LUNACRINE

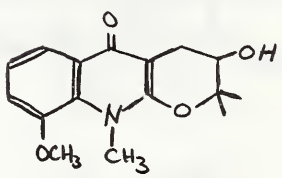


L. amara, (26, 27)

L. costulata, (24)

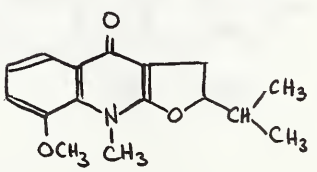
L. quercifolia, (32, 33)

LUNACRINOL



L. amara, (30)

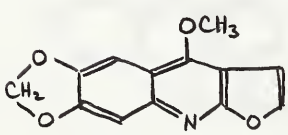
LUNINE



L. amara, (26)

L. quercifolia, (32, 33)

MACULINE

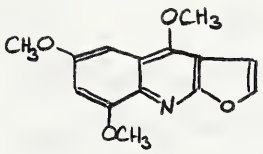


Fl. acuminata, (17)

Fl. maculosa, (19)

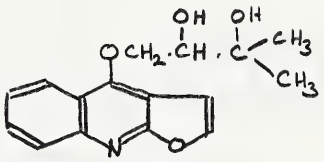
MACULOSIDINE

Fl. maculosa, (20)



MACULOSINE

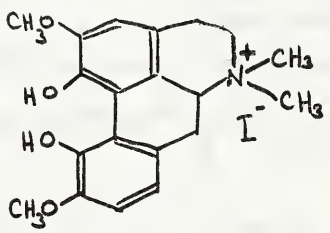
Fl. maculosa, (20)



MAGNOFLORINE

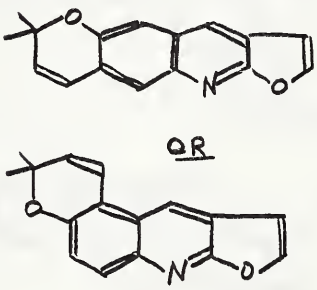
Magnolia grandiflora, (36)

Phellodendron amurense, (35)



MEDICOSMINE

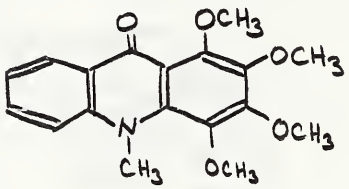
Medicosma cunninghamii, (21)



MELICOPICINE

A. bauerii, (4)

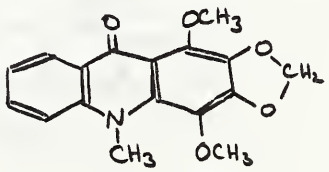
M. fareana, (3, 5)



MELICOPIDINE

A. bauerii, (4)

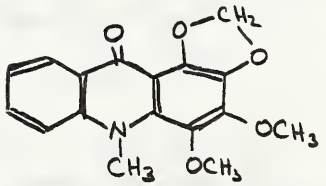
M. fareana, (3, 5)



MELICOPINE

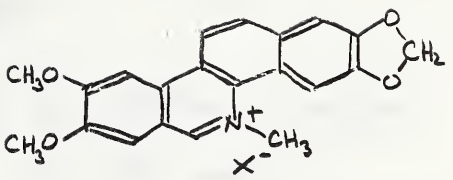
A. bauerii, (4)

M. fareana, (3, 5)



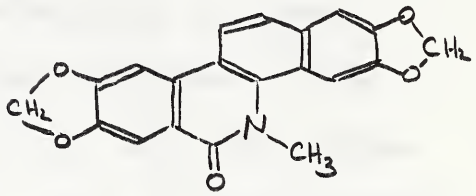
NITIDINE

X. nitidum, (41)



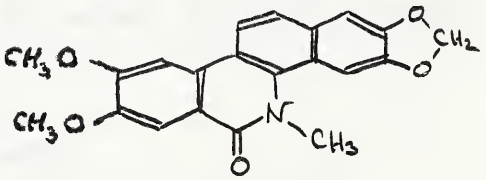
OXYAVICINE

X. avicennae, (42)



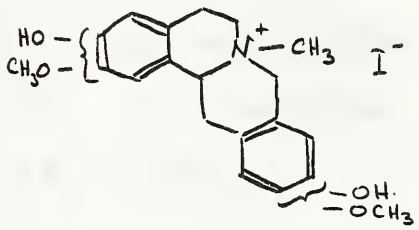
OXYNITIDINE

X. nitidum, (41)



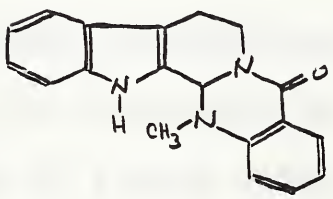
PHELLODENDRINE

P. amurense, (35, 37, 38)



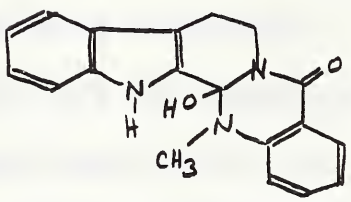
RHETSINE

X. rhetsa, (48, 49)



RHETSININE

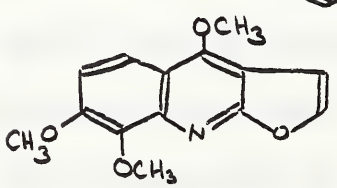
X. rhetsa, (48)



SKIMMIANINE

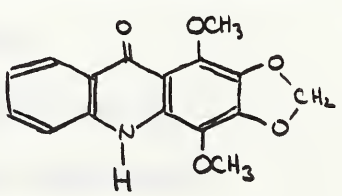
Skimmia repens, (6)

Many other Rutaceae



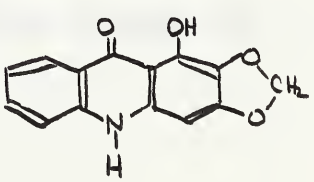
XANTHEVODINE

E. xanthoxyloides, (9)



XANTHOXOLINE

E. xanthoxyloides, (9)



DISCUSSION OF RESULTS

The previous investigations of the African species of Fagara by Paris and Moyse-Mignon (60, 61, 62) and by Paris and Palmer (62) showed that the alkaloids of these plants could be divided into four distinct classes on the basis of their chemical properties and their Rf. and ultra-violet fluorescence after separation by paper chromatography. These classes were defined thus:

A. Water soluble, quaternary bases.

B. Green fluorescent, Rf. $\doteq$ 0.59. e.g. angolinine.

C. Yellow fluorescent, Rf. $\doteq$ 0.62. e.g. angoline.

D. Blue or violet fluorescent, Rf. $\doteq$ 0.80. e.g. skimmianine.

Alkaloids of groups B and C are relatively strong bases, whereas those of group D are too weak to form salts in aqueous solution, even in the presence of strong acids. All of the African species so far examined have shown the presence of alkaloids representative of all of these groups.

The plan of the present investigation was to isolate alkaloids of the groups B, C and D and to examine in detail group B. With this in mind, the project was begun with an exploratory investigation of the alkaloids of the stem bark of F. melanacantha (Planch.) Eng.

In the light of previous work (66) on F. melanacantha it was expected that the two alkaloids melanacanthinine (group B) and melanacanthine (group C) would be the principal constituents.

Paper chromatography of the alkaloids from the ethanol extract showed the presence of bases corresponding to melanacanthinine and melanacanthine, but, although a small quantity of melanacanthinine was isolated, it was found that the major component was a previously unreported alkaloid. This substance, which was arbitrarily called "Base 4", showed a dark, non-fluorescent spot (Rf. = 0.76) on the paper chromatograms but only when present in relatively high concentration. This, coupled with the fact that the brown stain obtained by spraying with the modified Dragendorf reagent was very weak, may account for the fact that Base 4 was not detected in the previous work. In addition to Base 4, the bark was found to contain the known furoquinoline alkaloid, skimmianine (I), which is a common constituent of the Rutaceae but had not previously been reported in F. melanacantha.

Although the presence of melanacanthinine and melanacanthine was clearly indicated by paper chromatography, the separation and purification of these alkaloids proved to be extremely difficult. Small quantities of pure melanacanthinine were obtained, but attempts to purify melanacanthine were unsuccessful. Partition chromatography on columns of kieselguhr mixed with sodium phosphate/citric acid buffers of differing acidity was used in an attempt to separate the alkaloids. By a proper choice of buffer pH it was found possible to separate the alkaloids into two groups:

(a) Weak bases: Base 4 and skimmianine;

and (b) Strong bases: melanacanthinine and melanacanthine.

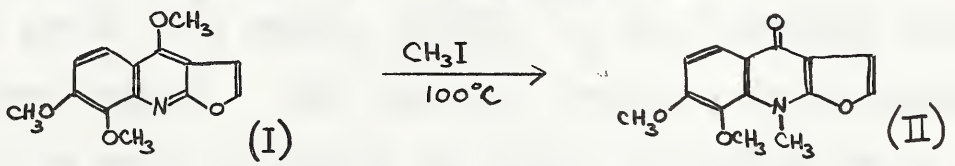
The weak base mixture, when rich in Base 4, yielded on concentration crystals of that material, but subsequent crops of crystals usually contained both Base 4 and skimmianine, and often further crystallisation was found to be impossible. Pure skimmianine (m.p. 177.5°C) was isolated from these mixtures by means of absorption chromatography on alumina.

A partial separation of melanacanthinine and melanacanthine was achieved by means of partition chromatography, using a buffer of pH 4.0. At this pH the melanacanthinine was held on the column until it was removed by eluting with ammoniacal chloroform whereas melanacanthine, together with some melanacanthinine, was eluted with chloroform. The crude melanacanthinine obtained by evaporation of the ammoniacal chloroform eluate was sometimes crystallisable but the melanacanthine fractions resisted all attempts to crystallise them. It was shown that chromatography on a more acid column (pH≏3.0) gave a better separation of the alkaloids but under these conditions crystallisation was rendered difficult due to considerable quantities of buffer which were eluted from the column along with the alkaloids. It was found that the small quantities of alkaloids in the eluate were not readily separable from the inorganic material.

Melanacanthinine, which, on paper chromatography, showed a green fluorescent spot (Rf. = 0.59), corresponds to the melanacanthinine previously isolated from the root bark of F. melanacantha by Paris and Palmer (66), and is a

representative of the "Group B" alkaloids. Unfortunately it was found to be present in insufficient quantities to warrent a structural investigation and work on it was therefore discontinued.

The new alkaloid, Base 4, was found by analysis to have the formula $C_{16}H_{15}NO_4$, and to contain one N-methyl and two methoxyl groups but no C-methyl groups. The Labat test for the methylenedioxy group was negative. It was soluble in chloroform, acetone, methanol and ethanol but insoluble in hydrocarbons, ether and pyridine. Because of its weakly basic character (pK_a = 3.4), its molecular weight and the position and colour of its spot on paper chromatograms, Base 4 was at first thought to belong to the furoquinoline group of alkaloids which so frequently occurs in the Rutaceae. However, its failure to react with methyl iodide to form an iso- compound, which is a general reaction of furoquinoline alkaloids, excluded this possibility. This reaction is illustrated for the case of skimmianine in (I)⟶(II).



The infra-red (I.R.) spectrum of Base 4 showed a peak at 1630 cm^{-1} which could be assigned to the lactam carbonyl group of a 2- or 4- quinolone. The absence of a peak at 940 cm^{-1} characteristic of the methylenedioxy group confirmed the negative Labat colour reaction. The ultra-violet (U.V.) spectrum showed a strong absorption at 274mμ which is in the range characteristic of a 4- quinolone. The formula, melting point and ultra-violet spectrum were similar

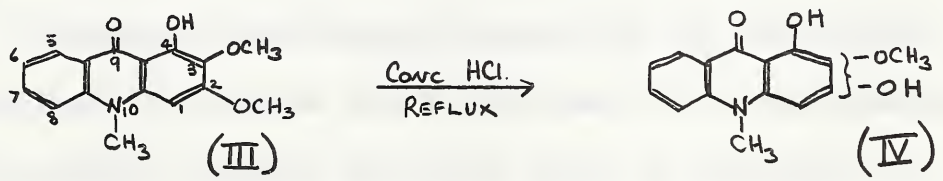
to those reported for the alkaloid arborinine which was obtained from Glycosmis arborea.

At the time of isolation of Base 4, the structure of arborinine was not known and so work on the elucidation of its structure was begun. After taking into consideration the two methoxyl groups, two oxygen atoms remained unaccounted for, although the peak at 1630cm^{-1} in the infra-red spectrum had been tentatively assigned to a lactam carbonyl group. Since the ultra-violet spectrum suggested the presence of a polycyclic system with extended aromatic conjugation, it seemed reasonable to suppose that the fourth oxygen atom was present in a phenolic hydroxyl group. The usual reactions for this group however, such as acetylation and methylation, all failed to take place. In view of this and since there was no hydroxyl stretching vibration in the infra-red spectrum (3600 - 3300cm^{-1}), it was apparent that if such a grouping were present it must be strongly bonded to the carbonyl group in a manner similar to the 4-hydroxy group in the acridone series. The base was found to be resistant to hydrolysis by acid or alkali but after refluxing for a prolonged period with concentrated hydrochloric acid a yellow crystalline product (m.p. 242°C), having the properties of a phenol, was obtained.

At this point further data on arborinine was published (71), showing that it was identical to 2,3-dimethoxy-4-hydroxy-10-methyl acridone (III) which had previously been isolated from Evodia xanthoxyloides (14), and it immediately became apparent that it was very similar, if not identical,

to Base 4. In particular, arborinine was reported (72) to give nor-arborinine (IV) (m.p. 242 - 243°C), by demethylation of one of the methoxyl groups, on refluxing with concentrated hydrochloric acid, and which was identical to a substance previously prepared from Base 4 by a similar reaction.

The identity of the Nuclear Magnetic Resonance (N. M. R.) spectra of Base 4 and arborinine and especially of the signals corresponding to the unique proton on C_1, which is flanked by nitrogen and oxygen atoms (4.10τ), and to the strongly hydrogen bonded phenolic proton, in the far downfield region (-4.7τ), placed beyond all doubt the identity of these two substances. The structure of Base 4 is therefore (III). This is the first reported occurrence of an acridone alkaloid in either of the genera Fagara or Xanthoxylum.



(III) (IV)

Returning to the original project, attention was now focussed on the species F. macrophylla, samples of the root bark of which had been received from Ghana. The bark was finely powdered and extracted by cold percolation, first with heptane to remove lipids and then with ethanol. The heptane extract did not contain any alkaloids but when concentrated was capable of producing an intense local anaesthesia on contact with the skin. Fagaramide, which had previously been isolated from this and other species of Fagara (50), was obtained from the heptane extract. Paper

chromatography of the crude ethanol extract showed the presence of alkaloids belonging to groups A, B, C and D, and, in addition, an alkaloid having a reddish-brown fluorescence (Rf.=0.60, i.e. between groups B and C).

Attempts to separate the alkaloids by means of partition chromatography were only partially successful. The Group B alkaloid was found to be almost insoluble in most organic solvents and this caused severe "trailing" on the columns which prevented a good separation. Small quantities of the Group B alkaloid were however obtained and its properties corresponded to those reported for xanthofagarine, which was isolated from this species by Paris and Moyse-Mignon (67). The yellow and red fluorescent alkaloids were not isolated in pure form in quantity and were not further investigated.

A process involving preparation of the crude alkaloidal hydrochlorides and partition of these between water and various organic solvents made it possible to separate the alkaloids into three main fractions:

(i) containing mainly xanthofagarine,

(ii) containing the yellow and red fluorescent alkaloids

and (iii) containing group D alkaloids (which are too weakly basic to form water soluble hydrochlorides), and neutral materials.

Examination of the fraction (iii) by absorption chromatography showed that it consisted mainly of fagaramide. A substance having a blue fluorescence and green phosphorescence (Rf. = 0.75) was detected in this fraction and will be mentioned later in this discussion.

Since xanthofagarine is a Group B alkaloid and since it was present in reasonably large quantities and was fairly readily extractable, this base was chosen for a further intensive study.

It was found more convenient to extract the finely powdered bark in a large soxhlet extractor, first with heptane and then with methanol. The methanol solution, on concentration, deposited a greenish-yellow amorphous solid which could be crystallised from glacial acetic acid. The product, which was found to be almost pure xanthofagarine, consisted of fine greenish-yellow needles. This material could be recrystallised from ethanol or methanol but always appeared to retain some solvent of crystallisation. Apparently for this reason, satisfactory analyses could not be obtained even after prolonged drying in vacuo.

Vacuum sublimation gave very pale yellow needles which did not depress the melting point when mixed with unsublimed material. Analysis of the sublimed sample gave the formula $C_{20}H_{15}NO_4$, with two methoxyl but no N-methyl or C-methyl groups. The Labat test for the methylenedioxy group was positive and a strong I.R. absorption peak at 940cm^{-1} was assigned to this grouping. Because of the difficulty involved in subliming large quantities of xanthofagarine, its reactions were investigated using unsublimed material.

Xanthofagarine was found to have low solubility in all solvents and this property made the planning and execution of reactions difficult. The best solvents were

glacial acetic acid, methanol, ethanol and water. Aqueous solutions could not be used in the presence of any acid other than acetic acid since the salts of xanthofagarine were very insoluble in the presence of even a slight excess of the parent acid. The same limitation applied to a somewhat lesser extent in alcoholic solutions. In all cases solution was difficult to effect and boiling with a large excess of the solvent was usually required.

A survey of the types of alkaloids previously isolated from the Rutaceae and having approximately the same molecular composition as xanthofagarine showed three main structural possibilities:

(i) The Aporphine skeleton,

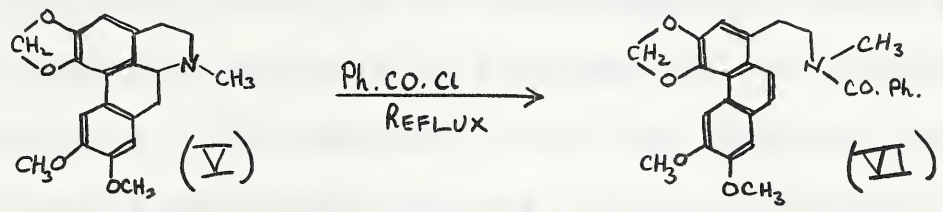
(ii) Berberine or Cryptopine skeletons

and (iii) the 1,2-Benzophenanthridine skeleton.

In addition there was some possibility that the molecule might contain an indole or carbazole structure. The Ehrlich test for the indole nucleus was however negative. All attempts to form an N-acetyl derivative met with complete failure and in most cases the starting material was recovered in good yield. No peak occurred in the infra-red spectrum in the N-H stretching region (3500 - 3300cm^{-1}). The fact that there does not appear to be an N-H group makes the indole and carbazole structures unlikely.

On treatment with boiling benzoyl chloride all known aporphine alkaloids undergo scission of the nitrogen containing ring to give an N-benzoyl compound. An illustration of this reaction is given for the alkaloid

dicentrine in (V) ⟶ (VI). Since xanthofagarine remained unchanged after prolonged treatment under these conditions it was concluded that the alkaloid was not of this type.



(V) (VI)

Since xanthofagarine had been found to be stable under a variety of conditions, though partial decomposition was observed under strongly alkaline conditions, it seemed likely that it possessed a complete stable aromatic system. This supposition was supported by N.M.R. evidence. An examination of the N.M.R. spectrum, the facilities for which had just become readily available, showed that xanthofagarine could not belong to the aporphine, cryptopine or berberine groups, since there were no signals corresponding to non aromatic protons, other than those contained in the substituent groups.

The N.M.R. spectrum of both sublimed and unsublimed xanthofagarine in trifluoroacetic acid showed two peaks of approximately equal area at 6.06τ and 6.18τ and these were assigned to the six protons of the methoxyl group. A two proton signal at 4.18τ was assigned to the methylenedioxy group. In addition the spectrum of the unsublimed material showed peaks at 5.42τ and 8.13τ which were initially assigned to the presence of water and acetic acid respectively in the sample. The assignment of the 8.13τ signal to acetic acid was confirmed by adding acetic acid to the sample and

re-scanning of the relevant portion of the spectrum. Integration of the signals in the aromatic region of the spectrum indicated a total of seven protons.

Catalytic reduction of xanthofagarine resulted in the smooth uptake of one mole of hydrogen and the solution became colourless. On exposure to air the solution rapidly became yellow and xanthofagarine was recovered in good yield. Sodium borohydride reduction gave similar results.

Oxidation under various conditions, both acidic and alkaline, gave rise to a neutral, colourless compound which was called oxyxanthofagarine. On paper chromatography this material gave a blue fluorescent spot (Rf. = 0.75) having a green phosphorescence and was thought to be the same material as that detected, along with fagaramide, in the weakly basic and neutral material fraction mentioned above. The N.M.R. spectrum of oxyxanthofagarine in trifluoroacetic acid showed two approximately equal peaks at 6.20τ and 6.28τ and these were assigned to two methoxyl groups. A two proton signal at 4.29τ was assigned to the two protons of the methylenedioxy group. Integration of the aromatic region indicated the presence of only six aromatic protons. A peak at 6.10 was not assigned. The infra-red spectrum of oxyxanthofagarine showed a peak at 1640cm^{-1} but was otherwise almost the same as that of xanthofagarine itself.

The behaviour of xanthofagarine on reduction was explained by assuming the presence of an aromatic system having one double bond which was susceptible to hydrogenation but easily re-oxidised to form the aromatic system. The

similar behaviour with sodium borohydride and the lack of colour in the reduction product suggested that the reaction might involve a carbon-nitrogen double bond in a fused aromatic system, since sodium borohydride does not usually reduce carbon-carbon double bonds.

From N.M.R. and analytical data it seemed likely that the formation of oxyxanthofagarine did not involve any extensive changes in the basic skeleton of the molecule. The peak at 1640cm^{-1} in the infra-red spectrum is in the region associated with lactam carbonyl groups and in particular with 2- and 4-quinolones and similar systems. An intense U.V. absorption at 287mμ is characteristic of 2-quinolones.

On the basis of the above evidence it was postulated that xanthofagarine possessed a fused aromatic system containing one nitrogen hetero-atom. Formation of oxyxanthofagarine was then assumed to proceed by oxidation of a carbon atom which is "α" to the nitrogen atom. From its molecular formula, spectral characteristics and reactions it seemed likely that xanthofagarine possessed a 1,2-benzophenanthridine structure. Alkaloids having this skeleton have only recently been isolated from some members of the Rutaceae.

A small scale zinc dust distillation of xanthofagarine yielded a mixture of products which could be separated by thin layer chromatography on silica gel. Although no pure materials were isolated, the chromatographic behaviour of the mixture was compared with that of several

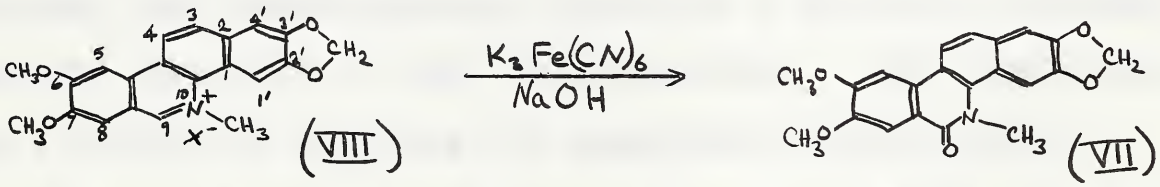
known substances and one of the components was found to behave very similarly to a sample of 5,7-dimethyl-1,2-benzophenanthridine. Since a sample of 1,2-benzophenanthridine itself was not readily available this investigation could not be carried any further.

In view of the fact that all of the benzophenanthridine alkaloids so far isolated have quaternary N-methyl groups it became necessary to critically re-evaluate the results of the present investigation of xanthofagarine. Since the quantitative analytical determinations of N-methyl groups in xanthofagarine were inconclusive and inconsistent it was decided to re-examine the N.M.R. spectral characteristics for indications of the presence of this group.

Whilst normally one would have expected an N-methyl group to give a peak in the range from 7.0τ to 7.8τ and a quaternary N-methyl group to give a signal in the region of 6.7τ, no such signals were observed in any of the xanthofagarine spectra. The signal at 5.42τ, however, which had been tentatively assigned to the presence of water in the sample, was found to have a uniform area, corresponding exactly to three protons, in independently prepared samples. This signal, in unsublimed xanthofagarine, could be assigned to the protons of a quaternary N-methyl group, which the absorption shifted considerably downfield. This shift was probably due to the presence of positively charged nitrogen and to the de-shielding effect of the polyaromatic system, enhanced by its coplanarity. The previously unassigned signal at 6.10τ in oxyxanthofagarine would then be due to the presence of the amide (lactam) N-methyl group.

The signal at 8.13τ in the spectrum of unsublimed xanthofagarine was due to the presence of acetic acid which was undoubtedly formed by replacement of the acetate ion by the ion of the stronger trifluoroacetic acid.

Concurrently it had been noticed that the U.V. spectrum and melting point reported for oxynitidine (VII), which was isolated from X. nitidum (41), corresponded closely to those of oxyxanthofagarine. Oxynitidine is a substituted benzophenanthridone obtained by oxidation of the related N-methyl-1,2-benzophenanthridine alkaloid: nitidine (VIII), also obtained from X. nitidum.



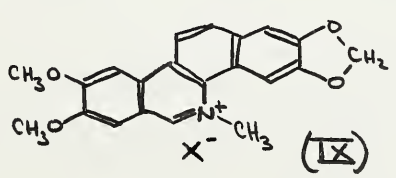
On heating, salts of nitidine were reported to undergo transformation to a compound, $C_{20}H_{15}NO_4$, having the same melting point as xanthofagarine. Authentic samples of oxynitidine and the pyrolysis product of nitidine were obtained* and a mixture of a sublimed sample of xanthofagarine and the pyrolysis product of nitidine was found not to show any depression of melting point. The infra-red spectra of these two materials were found to be identical in every respect.

*Gratefully received from H. R. Arthur, University of Hong Kong.

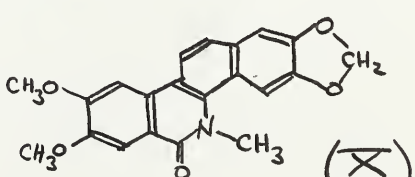
A mixed melting point of oxynitidine and oxyxanthofagarine showed a depression of 4°C, but it was found by means of thin layer chromatography on silica gel that the sample of oxynitidine contained four components, of which the major one resembled oxyxanthofagarine. Purification of the three milligram sample by thin layer chromatography was achieved but insufficient quantities of the pure sample were obtained to enable a comparable infra-red spectrum to be run. It was concluded that sublimed xanthofagarine is identical to the pyrolysis product of nitidine, and that oxyxanthofagarine and oxynitidine are identical.

On the basis of the evidence discussed above it was concluded that xanthofagarine possesses a quaternary N-methyl group and that this is lost on sublimation. The observation that a mixture of sublimed and unsublimed xanthofagarine showed no depression of melting point was due to the fact that demethylation of the unsublimed material occurred before the melting point was reached. The observed melting point of both materials is therefore in fact the melting point of the de-N-methylated substance.

Xanthofagarine, therefore, has the structure (IX), and oxyxanthofagarine the structure (X), and these compounds are identical to nitidine and oxynitidine respectively.



(IX)



(X)

It is suggested, on the basis of prior discovery (67), that the name "xanthofagarine" be retained for this compound. This is the first reported occurrence of a 1,2-benzophenanthridine alkaloid in an African species of Fagara, and it is expected that this work will provide the key to the elucidation of the structures of both the Group B and Group C alkaloids of the African Rutaceae.

EXPERIMENTAL SECTION

Part I - General

(a) Partition Chromatography

In all cases the support used in partition chromatography was "Hi-flo Supercel" - kieselguhr. The pH of the stationery phase was controlled by mixing numerically equal weights (in grams) and volumes (in millilitres) of "Hi-flo Supercel" and 1.0 molar disodium phosphate/citric acid buffers (73) of the required pH. The columns were packed in ether and the eluents normally used were, in order: ether, chloroform and chloroform saturated with ammonia (prepared by shaking chloroform with an equal volume of concentrated aqueous ammonia).

(b) Paper Chromatography

The progress of the separation of the alkaloids was followed by means of ascending paper chromatography. The composition by volume of the monophasic solvent used was:

Acetic acid (glacial)	3
isoAmyl alcohol	3
n-Heptane	1
Water (distilled)	2

The position of the components on the paper chromatograms was detected, first by examination of their fluorescence under ultra-violet light (2537Å and 3660Å), and then by spraying the chromatogram with the modified Dragendorf reagent (73) which gives a red-brown colouration with most of the alkaloids.

(c) Melting Points

All melting points were determined on a Leitz micro-Kofler block, using calibrated mercury thermometers.

(d) Spectral Data

Infra-red spectra were obtained on a Perkin Elmer DB 21 spectrophotometer. A Unicam SP 700, covering the U.V. and visible regions of the spectrum was used to obtain the Ultra-violet data. Nuclear Magnetic Resonance spectra were obtained on a Varian model A60 N.M.R. spectrometer, using tetramethyl-silane as an external reference standard.

(e) Thin Layer Chromatography

Glass plates (20cm. x 20cm., 20cm. x 10cm. or 20cm. x 5cm.) were coated with adsorbent in the usual manner to give a uniform 250μ layer, using a Desaga applicator. The adsorbent was Silica gel G supplied by Merck and Company, Darmstadt. Solvents consisting of mixtures of chloroform, n-heptane and methanol in different proportions, depending on the nature of the materials to be separated, were used. The separated materials were detected by their ultra-violet fluorescence, and by spraying with a 1% solution of ceric sulphate in 10% sulphuric acid, and heating in an oven at 110-120°C for five minutes.

Part II - Fagara Melanacantha

(a) Extraction

2.5kg. of the root bark of Fagara melanacantha (Planch.) Eng. was ground to a fine powder and exhaustively extracted with petroleum ether. The powder, after drying, was allowed to macerate for 24 hours in ammoniacal ethanol

and then extracted by cold percolation with a solvent consisting of 90% chloroform and 10% ethanol. The dark brown resinous material (70gm.) which remained after the solvent had been evaporated was dissolved in the minimum quantity of hot glacial acetic acid and the resulting solution was poured into five times its volume of cold water. After standing overnight the mixture was filtered through a kieselguhr pad. The liquid containing alkaloids in the form of their water soluble acetates was brought to neutrality with sodium hydroxide and then to pH 10 with ammonia and exhaustively extracted with chloroform. The aqueous liquors were made acid with hydrochloric acid and treated with excess of a saturated solution of ammonium reineckate to give a precipitate of the alkaloidal reineckates which was collected, washed with water and dried.

The precipitate of resins containing the alkaloids, obtained by pouring the hot acetic acid solution into water, was re-dissolved in glacial acetic acid and the process was repeated. This was continued until no further alkaloid could be extracted. Paper chromatography of the resinous residue showed the presence of Base 1 (melanacanthinine).

Total extracted of crude bases = 27.96gm. (1.08%)

Separation was followed by paper chromatography (see page 35). In the case of F. melanacantha the crude alkaloid extract showed six distinct spots under ultra-violet light.

	Fluorescence	Rf.	Dragendorf reaction	
(i)	green	0.37	brown	(Base 1)
(ii)	yellow	0.61	brown	(Base 2)
(iii)	green	0.62	-	
(iv)	dark	0.76	brown	(Base 4)
(v)	blue	0.84	pink	(Base 5)
(vi)	violet	0.94	-	

(b) Separation of the Alkaloids

(i) 12gm. of the crude chloroform extract were suspended in ether and placed on a 50gm., pH 5.0 partition column (No. 1). The distribution of bases in the eluates was:

fractions	1 - 30 (ether)	Bases 2, 4 and 5
	31 - 57 (ether)	Base 4 and some Base 5
	58 - 80 (chloroform)	Base 4 and some Base 1
	(chloroform - ammonia)	Base 1

Fractions 31 - 80 were bulked and crystallised from acetone/hexane mixture. Yield 350mg. of yellow needles of Base 4, m.p. 173 - 174°C.

Analysis, found: C, 66.87%; H, 5.28%; N, 5.03%

Calculated $C_{16}H_{15}NO_4$: C, 67.30%; H, 5.26%; N, 4.91%

Ultra-violet spectrum: λ_{max}: 274mμ; 400mμ.

(ii) A 440mg. portion of the ether fractions 5 - 25 from column No. 1 was placed on a 22gm. alumina column (activity III - IV on Brockman scale), and eluated with heptane, benzene, chloroform and methanol, in that order, with gradual changes of solvent. Base 4 appeared in most of the fractions. The distribution of alkaloids in the fractions was:

1 - 30, 20mg. (benzene and $CHCl_3$) Bases 2 and 4

30 - 40, 25mg. ($CHCl_3$ & 0.25% MeOH) Bases 2, 4 and 5

41 - 50, 80mg. ($CHCl_3$ & 0.5% MeOH) Base 5, some 2 and 4

51 - 80, 110mg. ($CHCl_3$ & 2.0% MeOH) Bases 1, 4 and 5.

Elution with methanol and then methanol/acetic acid mixtures removed traces of Base 1, but a bright yellow band, probably Base 1, remained firmly adsorbed.

Fractions 1 - 40 and 51 - 80 could not be crystallised and was processed later to yield Base 4. Fractions 42 - 50 were bulked and crystallised with difficulty giving 22mg. of brown cubes. This material was recrystallised from ethanol and 18mg. of pale amber crystals of Base 5 were obtained, m.p. 177 - 178°C. Mixed melting point with an authentic sample of skimmianine was 176 - 177.5°C.

Ultra-violet spectrum (in ethanol - not tested spectro grade): Base 5, λ_{max}: 249.5mμ; 320mμ; 332mμ.

Skimmianine : 249.0mμ; 330mμ.

(iii) 15.4gm. of crude chloroform extract were suspended in ether and placed on a 100gm., pH 5.0 partition column (No. 3). The distribution of bases was:

1 - 15 (ether) 6.5gm. Bases 1, 2, 4 and 5

16 - 83 (ether) 1.3gm. Base 4, with some 5 and 2

84 - 125 ($CHCl_3$) 3.3gm. Bases 4, 2 and 5

($CHCl_3/NH_3$) 0.55gm. Bases 1, 2, 4 and 5.

Selected fractions from the first few ether eluate fractions yielded, after two crystallisations from methanol, 2.5mg. of yellow needles of Base 1, m.p. 276 -

280°C. The remainder of the ether fractions yielded a total of 560mg. of Base 4, and 50 mg. of crude, semi-crystalline skimmianine.

(iv) The portions of fractions 1 - 20 (column No. 3) remaining after treatment as described in (iii) above, were dissolved in ether and placed on a 120gm., pH 4.8, partition column (No. 4).

1 - 10 (ether), 5.6gm. Bases 1, 2, 4, 5 and some oil
11 - end (ether & $CHCl_3$), 0.7gm. Bases 4, 2 and 5
$CHCl_3/NH_3$, 0.35gm. Base 1.

All the ether and chloroform fractions were un-crystallisable. The chloroform/ammonia eluate was dissolved in methanol and seeded with a crystal of melanacanthinine. After recrystallisation, 76mg. of pale yellow needles of Base 1 were obtained, m.p. 260 - 265°C.

Melting point of an authentic sample of melanacanthinine = 250 - 260°C.
Mixed melting point with Base 1: 246 - 260°C.

(v) 3.69gm. of crude chloroform extract obtained from the second extraction of resin material with acetic acid was found to contain mainly Bases 2 and 4. It was placed on a 120gm., pH 4.0, partition column (No. 5).

1 - 10 (ether), 1.6gm. Bases 2 & 4 with some 5 & oil
11 - 57 (ether), 1.5gm. Base 4 with some 5 and 2
58 - 100 ($CHCl_3$), 0.38gm. Bases 2 and 5
$CHCl_3/NH_3$), 0.15gm. Bases 1 and 2.

The early ether fractions were not crystallised but

fractions 15 - 57 crystallised to give 850mg. of Base 4. The chloroform fractions showed a predominance of Base 2 but crystallisation of this material could not be achieved. The chloroform/ammonia eluate contained mainly melanacanthinine (Base 1) but could not be crystallised.

(c) Reactions of Base 4.

(i) Determination of pK_a and molecular weight by titration

30mg. of Base 4 were dissolved in 15ml. of neutral ethanol and titrated in 0.1ml. steps with 0.01N. hydrochloric acid. The course of the titration was followed by means of a Beckman Model G pH meter. From the titration curve it was seen that Base 4 is a very weak base of $pK_a = 3.4$.

(ii) Attempted preparation of the hydrochloride of Base 4

The solution remaining after the pK_a determination was allowed to evaporate slowly at room temperature and the residue was recrystallised from ethanol. Yield, 23.5mg. yellow needles, m.p. 173 - 174°C, showing no depression of melting point when mixed with Base 4.

(iii) Preparation of the perchlorate of Base 4

50mg. of Base 4 were dissolved in methanol and the pH of the solution brought to 2.0 (universal paper) with 15% methanolic perchloric acid. The mixture was evaporated almost to dryness and recrystallised from acetone by freezing. After two crystallisations 13mg. of orange-yellow needles, m.p. 209 - 210.5°C were

obtained. The ultra-violet spectrum of this material showed the following peaks:

λ_{max}: 275mμ; 400mμ.

(iv) Attempted acid hydrolysis of Base 4

a. 20mg. of Base 4 was found to be insoluble in, and was recovered in 90% yield from, 3.0ml. of 5% aqueous hydrochloric acid after refluxing for 30 minutes.

b. The Base 4 recovered from a. was refluxed for 1 hour in a mixture of glacial acetic acid, water and concentrated hydrochloric acid (95 : 5 : 2). The mixture was diluted with cold water, neutralised with ammonia and extracted with chloroform.

Yield: 21mg. yellow needles, m.p. 172 - 174°C, showing no depression of melting point when mixed with Base 4.

(v) Attempted alkaline hydrolysis of Base 4

20.9mg. of Base 4 was refluxed with 3.0ml. of 20% potassium hydroxide in 90% aqueous ethanol for 30 minutes. The resulting mixture was acidified with hydrochloric acid, filtered to remove precipitated potassium chloride and evaporated. The residue was dissolved in chloroform washed with 5% potassium carbonate solution then with water and evaporated. The residue (22.1mg.) was crystallised from methanol.

Yield: 11mg. yellow needles, m.p. 172 - 175°C, showing no depression of melting point when mixed with Base 4.

Chromatograms of the mother liquor showed the presence of Base 4 only.

(vi) Reaction with methyl iodide

19.1mg. of Base 4 were placed in a sealed tube with 1.0ml. of methyl iodide and the tube was heated in steam for 5 hours. After cooling, the tube was opened and the product dissolved in chlorofrom, washed with 2N. aqueous ammonia then with water and evaporated to dryness. The residue (19.9mg.) was crystallised from methanol and gave 16mg. of bright yellow needles (m.p. 174 - 174.5°C). No depression of melting point occurred when the product was mixed with Base 4 and chromatograms of both the crystalline product and the mother liquor showed the presence of Base 4 only.

(vii) Hydrogenation of Base 4

10.1mg. of Base 4 was quantitatively hydrogenated in 2.0ml. of a solvent consisting of glacial acetic acid, water and concentrated hydrochloric acid, (95 : 3 : 2, by volume) and in the presence of 10.4mg. of Adam's catalyst (platinum oxide), at room temperature and atmospheric pressure. One mole of hydrogen was taken up in the first two hours and the reaction was then left for a further 15 hours when the final uptake of hydrogen was 2.01ml. Assuming a molecular weight of 285, this volume of hydrogen corresponded to an uptake of 2.2 moles of hydrogen. After filtration and evaporation 11.3mg. of almost colourless solid was produced. Recrystallisation from methanol yielded 7.8mg. of very pale yellow needles, m.p. 202.5 - 205°C.

U. V. spectrum (ethanol): λ_{max}: 254mμ; 340mμ.

(viii) Reaction of Base 4 with concentrated hydrochloric acid

68mg. of Base 4 were refluxed for 10 hours with 10ml. of concentrated hydrochloric acid. The alkaloid was initially insoluble in the acid but after about 1 hour a clear greenish-yellow solution was formed. The hot solution was quickly filtered and poured into an equal volume of cold water. The yellow precipitate was recrystallised from methanol giving 45mg. of yellow needles, m.p. 239 - 242°C. A second recrystallisation from methanol yielded 28mg. of crystals, m.p. 242.5 - 243°C. Unlike Base 4 this material was soluble in dilute (2N) sodium hydroxide giving a deep yellow-green solution.

Part III - Fagara Macrophylla

(a) Extraction (see Table IV)

(i) 6.8kg. of the root bark of F. macrophylla from Ghana was ground and exhaustively extracted by cold percolation first with heptane and then with ethanol. The golden yellow heptane extract on evaporation deposited 4gm. of a neutral white crystalline material ① , m.p. 117°C. Neither the precipitate nor the mother liquor gave alkaloidal reactions with Meyer's or Dragendorf's reagents.

During evaporation, the dark brown ethanol extract deposited a total of 4gm. of yellow/orange solid ③. The dark brown sticky residue which remained when most of the ethanol had been evaporated was dissolved in the minimum amount of hot glacial acetic acid and the

TABLE IV

F. MACROPHYLLA COLD PERCOLATION FLOW SHEET

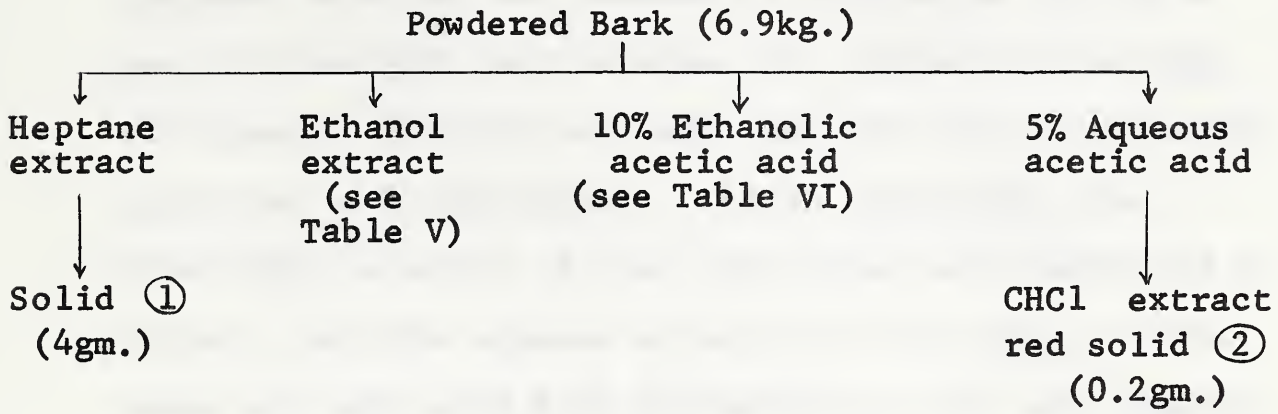
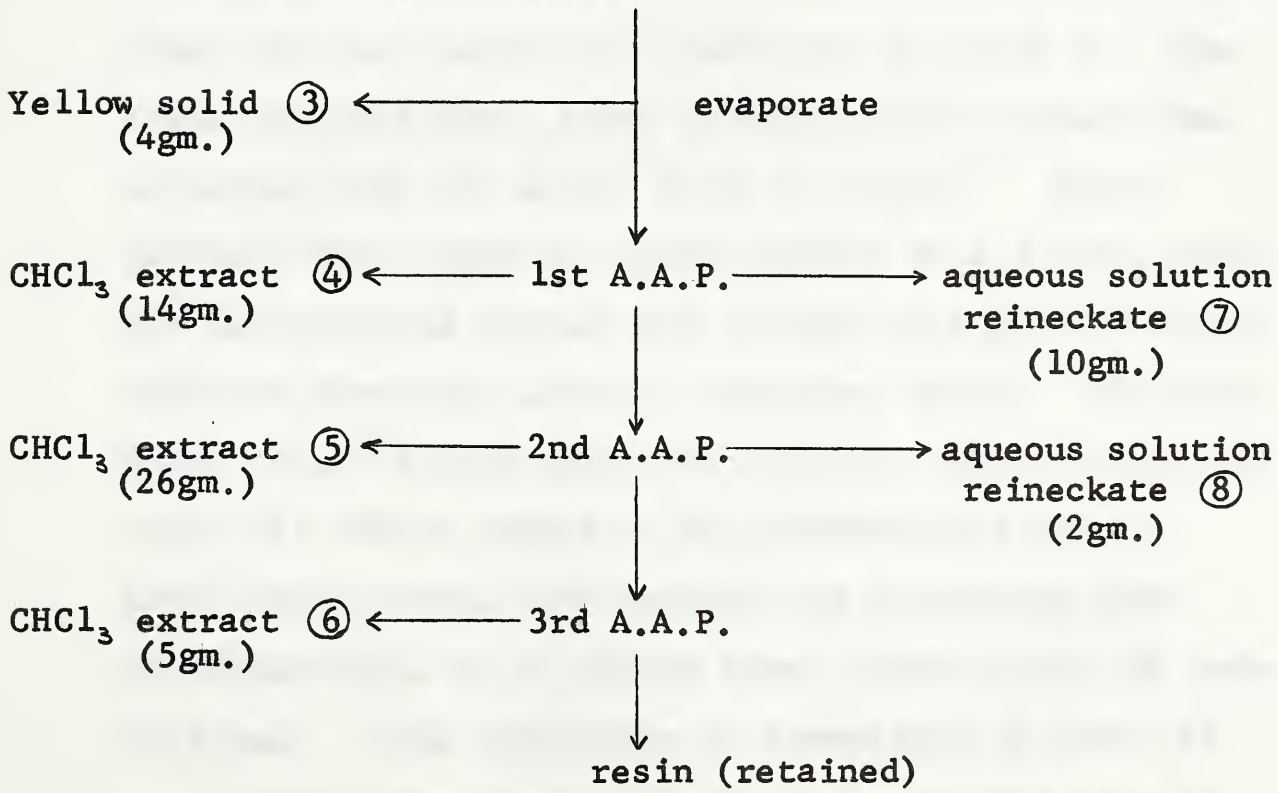


TABLE V

ETHANOL EXTRACT FLOW SHEET



resulting solution poured into five times its volume of cold water. After standing overnight the precipitated resinous material was removed by filtration through a pad of kieselguhr and retained for further processing. The aqueous filtrate was made alkaline with ammonia and extracted with chloroform. After separation, the chloroform solution of the crude bases was evaporated to dryness, and the aqueous solution of the water soluble bases was made acid with hydrochloric acid, and treated with excess of a saturated solution of ammonium reineckate, to precipitate the alkaloids in the form of their reineckates. The above process, which was called the acetic acid process and designated "A. A. P." for convenience, was repeated on the precipitated resin several times and the results are summarised in Table V. The crude powdered bark, after extraction with ethanol was extracted with 10% acetic acid in ethanol. These extracts were found to contain mainly Base 1 (see later). The concentrated extract was treated with glacial acetic acid and then with water as described above. The dark brown acidic liquid deposited a yellow semi-crystalline solid 11 which proved to be predominantly Base 1. After basification with ammonia and extraction with chloroform 4gm. of a reddish brown sticky solid ⑨ were obtained. This separation is summarised in Table VI.

Finally the plant material was extracted with 5% aqueous acetic acid. The resulting black foaming liquid was made alkaline with ammonia and extracted with

TABLE VI

ACETIC ACID/ETHANOL EXTRACT FLOW SHEET

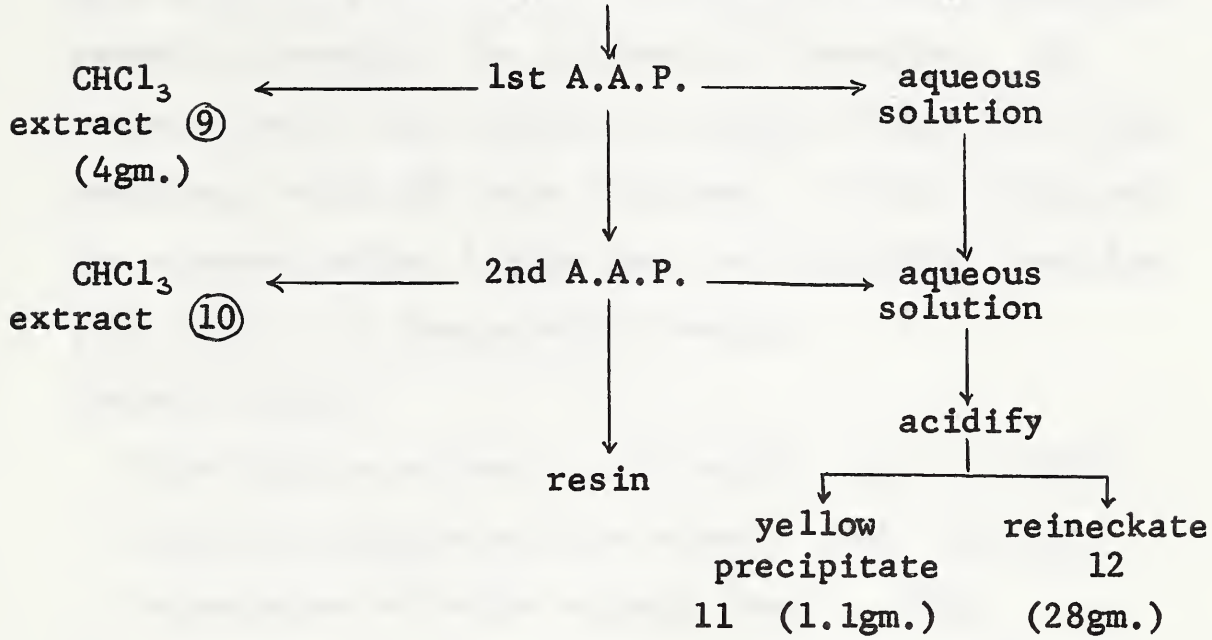


TABLE VII

PRINCIPAL CONSTITUENTS OF THE FRACTIONS DESCRIBED IN TABLES IV - VI

1. Fagaramide
2. Unknown
3. Base 1
4. Bases 1 and 4, fagaramide
5. Fagaramide, Bases 1 and 4
6. Bases 1, 2 and 3
9. Bases 1, 3 and 4 and fagaramide
10. Bases 1 and 4
11. Base 1.

chloroform. The extraction was made difficult by the fact that very persistant emulsion formation occurred, probably formed by the presence of saponins. On evaporation of the chloroform extract 0.2gm. of a red amorphous solid ② were obtained. Neither this nor the aqueous mother liquor gave an alkaloidal reaction with Meyer's or Dragendorf's reagent.

Overall yields:

from Heptane extract: dry solid - 4gm. (0.059%)

crude chloroform soluble bases: 58gm. (0.85%)

reineckates of water soluble bases: 40gm.

(ii) Approximately 1kg. lots of the powdered bark were extracted with heptane for about 36 hours, and then with methanol for about 3 days in a soxhlet extractor. The nature and yield of the material obtained by soxhlet extraction, with one important exception, differ little from those obtained by cold percolation, but the extraction by soxhlet was found to be much less time consuming. The yield of Base 1, which was found to be rather insoluble in most solvents, was much greater in soxhlet extraction than in the cold percolation, since in the latter case much of the Base 1 remained in the bark until it was extracted with ethanol/acetic acid. Most of the material used in the following practical work was obtained by soxhlet extraction.

As in the case of F. melanacantha the course of the separation was followed by means of paper chromatography. The crude alkaloidal extract showed five distinct spots.

	Fluorescence	Rf.	Dragendorf reaction	
(i)	green	0.57	brown	(Base 1)
(ii)	red-brown	0.59	red	(Base 2)
(iii)	yellow	0.61	brown	(Base 3)
(iv)	blue	0.83	faint	(Base 4)
(v)	deep blue	0.92	-	

(b) Separation of the Alkaloids

(i) The neutral, white crystalline material ① which was precipitated from the concentrated heptane extracts was recrystallised from ethyl acetate several times. The product, m.p. 117.5°C gave a deep blue spot (Rf. = 0.92) on the paper chromatograms. The crude material was purified also by sublimation in vacuo at 100°C. The sublimate, m.p. 117.5°C gave no depression of melting point when mixed with the unsublimed material and the I.R. spectra of the sublimed and unsublimed samples were identical. The Labat test for the methylenedioxy group was positive.

I. R. absorption peaks were assigned thus:

3250cm^{-1} (strong)	N-H stretching
1650cm^{-1} (strong)	amide carbonyl group
940cm^{-1}	methylenedioxy group.

Analysis found: C, 68.08%; H, 6.98%; N, 5.62%.

Calculated for $C_{14}H_{17}NO_3$: C, 68.05%; H, 6.89%; N, 5.67%.

The above data correspond exactly to that of fagaramide which has several times been isolated from this species (50, 67).

(ii) 1.5gm. of the yellow material ③ which was precipitated from the crude methanol extract on evaporation was crystallised from glacial acetic acid and gave 175mg. of greenish-yellow needles, m.p. 278 - 282°C of Base 1, showing no depression of melting point when mixed with a sample of Xanthofagarine.

(iii) 9.5gm. of the crude chloroform extract were suspended in ether and placed on a 500gm. pH 5.7 partition column (No. 6). The starting material showed chromatographic spots corresponding to Bases 1, 2, 3 and 4 and also fagaramide. The distribution of these substances in the eluate was as follows:

ether, (5.4gm.), Bases 1, 2, 3, 4 and fagaramide

$CHCl_3$, (0.95gm.), Bases 1, 2 and 3

$CHCl_3/NH_3$, (1.1gm.), Bases 1 and 3.

None of the fractions yielded crystalline material.

(iv) The ether eluate from the above column (No. 6) was dissolved in chloroform, treated with ether which had been saturated with dry hydrogen chloride and the mixture was evaporated to dryness. The residue was shaken with a mixture of water and chloroform, and thus separated into two fractions.

a. Water soluble hydrochlorides, Bases 2 and 3

b. Chloroform soluble materials, Bases 4 and 1, and fagaramide.

(v) The water soluble fraction a. was made alkaline with ammonia, extracted with chloroform and the chloroform layer evaporated to dryness yielding 0.6gm.

of a mixture of Bases 2 and 3 with only traces of Base 4. Crystallisation from ethanol gave 40mg. of poor quality yellow crystals of Base 3, m.p. 175°C.

(vi) The chloroform fraction b. from column No. 6 was treated with more ether/HCl and the above process, (iv), was repeated until no precipitate was produced when the ether/HCl was added to the chloroform solution. The chloroform solution was then added to the chloroform eluate from column No. 6 and evaporated to dryness.

A 0.99gm. portion of this mixture was placed on a 25gm. pH 4.4 partition column (No. 7). The bases were distributed in the fractions as follows:

1 - 2 (ether), 314mg., Bases 3 & 4, traces of 1 & 2

3 - 8 (ether), 152mg., Bases 3, 2 and 4

9 - end ($CHCl_3$), 46mg., Bases 2, 3 and 4

$CHCl_3/NH_3$, 54mg., Base 1, traces of Base 3.

Some yellow brown material remained on the column.

(vii) Separation of the alkaloids by preparation of the hydrochlorides

This process was applied to many of the crude alkaloid samples obtained from the columns described above. The mixture of materials was dissolved in chloroform and an excess of ether saturated with dry hydrogen chloride was added, forming a brown-yellow precipitate of alkaloid hydrochlorides. The mixture was evaporated to dryness and shaken with a mixture of chloroform and water. The aqueous layer was made alkaline and extracted with chloroform. Evaporation of the chloroform layer gave fraction B. The yellow

aqueous layer was made acid with hydrochloric acid and extracted with chloroform or n-butanol and evaporation of this organic extract gave fraction C. The chloroform soluble hydrochlorides and neutral materials which were not extracted from chloroform into the water in the first extraction constitute fraction A.

Although pure materials were not in general obtained by this method, a useful separation of groups is achieved.

Fraction A: Base 4 and fagaramide

B: Bases 2 and 3

C: Base 1, some Base 3.

The results of some separations by this method are indicated below. The numbers refer to the fractions obtained from the crude alkaloidal extract (see Tables V, VI and VII).

Fractions 9 and 10, (4.2gm.):

A: 0.70gm., Base 4 and fagaramide

B: 2.14gm., Bases 1 and 3

C: 0.28gm., Base 1, traces of Base 3.

3.12gm.

Fractions 5 and 6, (31gm.):

A: 18.95gm., Base 4 and fagaramide

B: 2.64gm., Bases 2 and 3

C: 1.20gm., Bases 1 and 3.

22.79gm.

(viii) Preparation of Base 3

Fractions of type B containing Base 3 were dissolved in chloroform saturated with ammonia, and the

pale pink solution was evaporated to dryness. The residue was dissolved in ethanol and two crops of yellow crystals were obtained, which were chromatographically pure Base 3, m.p. 246 - 247°C.

(ix) Preparation of xanthofagarine

a. Fractions of type C which contained predominantly Base 1 were dissolved in methanol and recrystallised, yielding a total of 265mg. of pure xanthofagarine, m.p. 277 - 278°C.

Separation of xanthofagarine from mixtures with the other bases by partition chromatography was not found to be conveniently achievable.

b. The methanol extracts from the soxhlet extraction of the root bark was slowly evaporated in several stages and at each stage a yellow precipitate was found to be formed. This was filtered off, boiled with water and filtered. The filtrate was treated with concentrated hydrochloric acid and the greenish precipitate of water insoluble hydrochlorides filtered off and recrystallised from glacial acetic acid, yielding crystalline xanthofagarine, chromatographically pure, m.p. 269 - 270°C. The xanthofagarine was recrystallised from its methanolic solution to which a few drops of aqueous ammonia had been added. This method was found to be convenient for the preparation of relatively large amounts of xanthofagarine and was used for most of the xanthofagarine used in the investigation of its reactions.

The precipitate from the methanol extracts became browner as the solutions became more concentrated and the material obtained from these by the above process was found to be rich in Base 2. This observations may be a clue to the isolation of Base 2 but was not further investigated in this project.

(c) Properties and Reactions of Xanthofagarine

(i) Xanthofagarine is soluble in glacial acetic acid, water, methanol, ethanol and butanol and almost insoluble in chloroform, acetone, ether and hydrocarbons. It melts at 278.5 - 279.5°C and sublimes easily in vacuo at 200°C giving almost colourless needles, m.p. 283°C, and showing no depression of melting point when mixed with an unsublimed sample.

Analysis of the sublimed sample:

Found: C, 71.93%; H, 4.56%; N, 4.35%; O (direct), 19.20%.

Calculated for $C_{20}H_{15}NO_4$: C, 72.07%; H, 4.51%; N, 4.21%; O, 19.22%.

Found: O-CH_3, 12.75%; N-CH_3, 2.92%.

Calculated: $(O\text{-}CH_3)_2$, 18.6%; N-CH_3, 9.73%.

The Labat test for the methylenedioxy group, carried out by dissolving a crystal of the material in a freshly prepared solution of gallic acid in concentrated sulphuric acid was positive (appearance of a blue colour) for both sublimed and unsublimed materials.

(ii) Spectral data

The N.M.R. spectrum of unsublimed xanthofagarine

showed the following peaks: 8.13τ, (3 H); 6.18τ, (3 H); 6.06τ, (3 H); 5.42τ, (3 H); 4.18τ, (2 H); aromatic proton range, 1.7τ- 3.0τ, (7 H). The N.M.R. spectrum of the sublimed material was identical except that the peaks at 8.13τ and 5.42τ were missing.

The U.V. absorption maxima for the sublimed sample of xanthofagarine were:

λ_{max}, (ε); 229.9mμ,(35,380); 280.1mμ,(33,320); 310.6mμ,(18,690).

No shift occurred in alkaline solution but after the addition of a drop of acid the observed maxima were:

λ_{max}, (ε); 220.8mμ,(19,510); 237.0mμ,(20,650); 271.8mμ,(40,340); 291.5mμ,(40,340); 300.3mμ,(38,630); 328.9mμ,(38,740); 387.6mμ,(10,320).

(iii) Ehrlich test

1mg. of xanthofagarine was dissolved in 1ml. of hot methanol and one drop of a solution of p-dimethylamino-benzaldehyde was added. An immediate precipitate of the alkaloidal hydrochloride appeared but no blue colour, indicative of the presence of an indole nucleus, was observed.

(iv) Attempted acetylation of xanthofagarine

A mixture of 40mg. of xanthofagarine, 8ml. of acetyl chloride and 1ml. of pyridine was refluxed for 1½ hours. The hot mixture was poured into cold water made alkaline with potassium carbonate and extracted with chloroform. The chloroform extract was washed with water and evaporated to dryness leaving 30mg. of yellow solid.

The material was contaminated with pyridine which was not removed, but chromatograms of the product showed the presence of xanthofagarine only.

(v) Catalytic hydrogenation of xanthofagarine

20mg. of xanthofagarine was quantitatively hydrogenated in the presence of 11.3mg. of Adam's catalyst (platinum oxide) in 4ml. of 2% methanolic acetic acid, at a temperature of 20°C and a pressure of 690.9mm. 1.53ml. of hydrogen were taken up smoothly in ten minutes and the solution became colourless. The hydrogen uptake corresponded to 0.96 mole-equivalents on the basis of a molecular weight of 333 for xanthofagarine. On opening the reaction flask to the atmosphere the mixture rapidly became yellow and after recrystallisation from methanol 17mg. of yellow crystals were obtained which were found not to depress the melting point of a sample of xanthofagarine and which showed only the xanthofagarine spot on the paper chromatograms. It was not found convenient to isolate the dihydroxanthofagarine which was presumed to be formed on hydrogenation.

(vi) Sodium borohydride reduction of xanthofagarine

41.9mg. of xanthofagarine were dissolved in 5ml. of methanol and a solution of 22mg. of sodium borohydride in 5ml. of methanol was added slowly over a period of ten minutes. There was some effervescence and the solution became colourless. Stirring was continued for one hour after the addition was complete and then 5ml. of 2N methanolic hydrochloric acid and refluxed for ten minutes.

The resulting colourless liquid was evaporated to small volume and extracted with chloroform. The chlorofrom extract rapidly turned yellow in air and, as in the case of the catalytic hydrogenation of xanthofagarine, only xanthofagarine itself could be isolated from the reaction mixture.

(vii) Preparation of oxyxanthofagarine

a. A solution of 210mg. of xanthofagarine in 375ml. of 9% acetic acid in acetone was prepared by dissolving the alkaloid in the requisite quantity of hot glacial acetic acid and diluting with acetone. 153mg. of finely powdered potassium permanganate was added in approximately 20mg. portions, at room temperature, over a period of three hours. The mixture was allowed to stand for one hour before destroying excess reagent with sulphur dioxide. The mixture was then evaporated almost to dryness and taken up in a mixture of water and chloroform. The chloroform layer was extracted with 10% aqueous bicarbonate solution and the alkaline extract was made acid with hydrochloric acid and extracted with chloroform yielding 3mg. of a yellow solid.

The chloroform solution of the neutral oxidation products was placed on a 3gm. alumina column (grade III). Elution with benzene and benzene containing 10% chloroform yielded 50mg. of a colourless solid which after recrystallisation from chloroform/methanol showed a blue fluorescent spot (Rf. = 0.75) having a green phosphorescence, m.p. 278.5 - 280°C. This product, which

was called oxyxanthofagarine, was found to sublime readily in vacuo at 200°C and the I.R. spectra of the sublimed and unsublimed samples were found to be identical.
Sublimed sample m.p. 281 - 281.5°C.
λ_{max} (ethanol), (log ϵ): 250.6mμ, (4.52); 280.1mμ, (4.60); 287.4mμ, (4.65); 320.5mμ, (4.12); 334.4mμ, (4.12); 366.3mμ, (3.67).
No acid or base shifts were observed in the U.V. absorption maxima.
N.M.R. signals occur at: 6.28τ, (3 H); 6.20τ, (3 H); 6.10τ, (3 H); 4.29τ, (2 H); and aromatic proton region, 1.7τ- 3.0τ, (6 H).

b. 400mg. of xanthofagarine was dissolved in 150ml. of boiling water and 1% potassium hydroxide solution was added drop by drop until the alkaloid showed signs of precipitation. 3% aqueous potassium permanganate solution was added in 5ml. portions at room temperature, with vigorous mixing. A total of 50ml. of potassium permanganate solution was added over a period of three hours, and the mixture was allowed to stand for a further 1½ hours. By the same method to that described above, 120mg. of oxyxanthofagarine were obtained.

c. 52mg. of xanthofagarine were dissolved in 10ml. of water and a hot solution of 200mg. of potassium ferricyanide and 90mg. of potassium hydroxide in 5ml. of water was added. A white precipitate formed immediately and the mixture was warmed on a steam bath for 15 minutes

then cooled and extracted with chloroform. On evaporation of the chloroform extract and crystallisation of the residue 45.2mg. of oxyxanthofagarine were obtained.

(viii) Zinc dust distillation of xanthofagarine

108mg. of xanthofagarine were intimately mixed with 500mg. of zinc dust and the mixture heated in a sealed tube under vacuum for 48 hours at 300°C. The residue was extracted in turn with benzene, chloroform and methanol. The extracts were separately evaporated and the following weights of residue were obtained:

Benzene	3.6mg.
Chloroform	2.4mg.
Methanol	29.9mg.

(ix) Thin layer chromatography of the zinc dust distillation products

Using a solvent consisting of chloroform (7ml.), n-hexane (13ml.) and methanol (0.2ml.) and under the general conditions outlined on page 36, it was found possible to separate quantitatively the products of the zinc dust distillation. The major component of the methanol extract, under these conditions, had an Rf. of 0.4 and had a blue fluorescence. Using the same conditions, the behaviour of various substances such as quinoline, naphthalene and phenanthrene was compared with that of the zinc dust distillation products. It was found that the substance which most closely resembled the major product, not only in Rf. and colour of fluorescence, but also in its behaviour when the

solvent system was changed, was 5,7-dimethyl-1,2-benzophenanthridine.

(x) Purification of oxynitidine by thin layer chromatography

A solvent consisting of chloroform (6ml.), n-hexane (12ml.) and methanol (3.3ml.) was found to be satisfactory for the separation of oxynitidine from the impurities present in the sample. The 3.5mg. sample was dissolved in chloroform and placed in a band along the bottom of a thin layer plate. The plate was developed and the position of the oxynitidine band was noted under ultraviolet light. The silica gel in the region of the oxynitidine spot was removed from the plate by suction and collected in a micro soxhlet thimble. Extraction of this with chloroform, and evaporation of the extract gave 1.75mg. of chromatographically pure oxynitidine. The chromatographic characteristics of this were identical to those of oxyxanthofagarine.

BIBLIOGRAPHY

1. F. N. Lahey, W. C. Thomas. Aust. J. Sci. Res. A2, 423 (1949); through Chem. Abstr. 45, 5696d (1951).
2. F. N. Lahey, J. A. Lamberton, J. R. Price. Aust. J. Sci. Res. A3, 155 (1950); through Chem. Abstr. 46, 4016a (1952).
3. J. R. Price. Aust. J. Sci. Res. A2, 249 (1949); through Chem. Abstr. 46, 4010d (1952).
4. J. A. Lamberton, J. R. Price. Aust. J. Chem. 6, 66 (1953).
5. W. D. Crow, J. R. Price. Aust. J. Sci. Res. A2, 282 (1949); through Chem Abstr. 46, 4014a (1952).
6. Y. Asahina, M. Inibuse. Ber. dtsch. chem. Ges. 63, 2052 (1930).
7. R. A. L. Anet, P. T. Gilham, P. Gow, G. K. Hughes, E. Ritchie. Aust. J. Sci. Res. A5, 412 (1952); through Chem. Abstr. 47, 3860g (1953).
8. G. K. Hughes, K. G. Neill, E. Ritchie. Aust. J. Sci. Res. A5, 401 (1952); through Chem. Abstr. 47, 3857f (1953).
9. J. R. Cannon, G. K. Hughes, K. G. Neill, E. Ritchie. Aust. J. Sci. Res. A5, 406 (1952); through Chem. Abstr. 47, 3858a (1953).
10. F. W. Eastwood, G. K. Hughes, E. Ritchie. Aust. J. Chem. 7, 87 (1954).
11. R. H. Prager, E. Ritchie, A. V. Robertson, W. C. Taylor. Aust. J. Chem. 15, 301 (1962).

12. R. G. Cooke, H. F. Haynes. Aust. J. Chem. 7, 273 (1954).

13. H. Thoms. Ber. dtsch. pharm. Ges. 33, 68 (1923); through Chem. Abstr. 17, 2583 (1923).

14. R. J. Gell, G. K. Hughes, E. Ritchie. Aust. J. Chem. 8, 114 (1955).
G. K. Hughes, K. G. Neill. Aust. J. Sci. Res. A2, 429 (1949); through Chem. Abstr. 46, 117b (1952).

15. H. Matthes, E. Schreiber. Ber. dtsch. pharm. Ges. 24, 385 (1914).

16. R. F. C. Brown, J. J. Hobbs, G. K. Hughes, E. Ritchie. Aust. J. Chem. 7, 348 (1954).

17. E. Ritchie, W. C. Taylor, S. T. K. Vautin. Aust. J. Chem. 14, 469 (1961).

18. S. V. Binns, B. Halpern, G. K. Hughes, E. Ritchie. Aust. J. Chem. 10, 480 (1957).

19. R. J. Gell, G. K. Hughes, E. Ritchie. Aust. J. Chem. 8, 422 (1955).

20. R. H. Prager, E. Ritchie, W. C. Taylor. Aust. J. Chem. 13, 380 (1960).

21. J. A. Lamberton, J. R. Price. Aust. J. Chem. 6, 173 (1953).

22. J. R. Cannon, G. K. Hughes, E. Ritchie, W. C. Taylor. Aust. J. Chem. 6, 86 (1953).

23. H. F. Haynes, E. R. Nelson, J. R. Price. Aust. J. Sci. Res. A5, 387 (1952); through Chem. Abstr. 47, 3858f (1953).

24. W. G. Boorsma. Bull. Inst. Bot. Buitenzorg 6, 15 (1900).

25. F. A. Steldt, K. K. Chen. J. Am. Pharm. Assoc., 32, 107 (1943); through Chem. Abstr. 37, 3562^{4} (1943).

26. S. Goodwin, J. N. Shoolery, L. F. Johnson. J. Am. Chem. Soc., 81, 3065 (1959).

27. S. Goodwin, F. C. Horning. J. Am. Chem. Soc., 81, 1908 (1959).

28. S. Goodwin, A. F. Smith, A. A. Velasquez, E. C. Horning. J. Am. Chem. Soc., 81, 6209 (1959).

29. S. Goodwin, J. N. Shoolery, E. C. Horning. J. Am. Chem. Soc., 81, 3736 (1959).

30. H. C. Beyerman, R. W. Rooda. Koninkl. Ned. Akad. Wetenschap. Proc. Ser. B.63, 154 (1960); through Chem. Abstr. 54, 18573b (1960).

31. H. C. Beyerman, R. W. Rooda. Koninkl. Ned. Akad. Wetenschap. Proc. Ser. B.62, 187 (1959); through Chem. Abstr. 54, 2393d (1960).

32. R. Johnstone, J. R. Price, A. R. Todd. Aust. J. Chem. 11, 562 (1958).

33. J. R. Price. Aust. J. Chem. 12, 458 (1959).

34. Y. Murayama, K. Shinozaki. J. Pharm. Soc. Japan, No. 330, 299 (1936); through Chem. Abstr. 21, 2049 (1927).

35. M. Tomita, T. Nakano. Pharm. Bull. (Tokyo) 5, 10 (1957); through Chem. Abstr. 51, 15063d (1957).

36. T. Nakano. Pharm. Bull. (Japan) 2, 329 (1954); through Chem. Abstr. 50, 7117h (1956).

37. M. Tomita, J. Kunitomo. Yakugaku Zasshi 80, 880 (1960); through Chem. Abstr. 54, 24828c (1960).

38. M. Tomita, J. Kunitomo. Yakugaku Zasshi 80, 885 (1960); through Chem. Abstr. 54, 24829b (1960).

39. M. Tomita, J. Kunitomo. Yakugaku Zasshi, 78, 1444 (1958); through Chem. Abstr. 53, 7219a (1959).

40. M. Tomita, H. Ishii. Yakugaku Zasshi, 79, 1228 (1959); through Chem. Abstr. 55, 1677h (1961).

41. H. R. Arthur, W. H. Hui, Y. L. Ng. J. Chem. Soc. 1959 1840.

42. H. R. Arthur, W. H. Hui, Y. L. Ng. J. Chem. Soc. 1959 4007.

43. H. R. Arthur, Y. L. Ng. J. Chem. Soc. 1959, 4010.

44. K. W. Gonipath, T. R. Govindachari, P. C. Parthasarathy, N. Viswanathan. J. Chem. Soc. 1959, 4012.

45. P. J. Scheuer, M. Y. Chang, C. E. Swanholm. J. Org. Chem. 27, 1472 (1962).

46. H. R. Arthur, H. T. Cheung. Aust. J. Chem. 13, 510 (1960).

47. H. R. Arthur, L. Y. S. Loh. J. Chem. Soc. 1961, 4360.

48. A. Chatterjee, S. Bose, C. Ghosh. Tetrahedron 7, 257 (1959).

49. K. W. Gonipath, T. R. Govindachari, V. Ramados-Rao. Tetrahedron 8, 293 (1960).

50. J. A. Goodson. Biochem. J. 15, 123 (1921).

51. C. E. Redeman, B. B. Wisegarver, G. A. Alles. J. Am. Chem. Soc. 71, 1030 (1949).

52. G. V. Stuckert. Investigaciones del Laboratorio de Quimica Biologica de Cordoba, Argentina, 1, 94 (1933); through reference 51.

53. V. Deulofeu, R. Labriola, J. de Lanche. J. Am. Chem. Soc. 64, 2326 (1942).

54. B. Berinzaghi, A. Muruzabal, R. Labriola, V. Deulofeu. J. Org. Chem. 10, 81 (1945).

55. G. Comin, V. Deulofeu. J. Org. Chem. 19, 1774 (1954).

56. G. Comin, V. Deulofeu. Tetrahedron 6, 63 (1959).

57. L. D. Antonaccio. An. Acad. Brasil Ciênc 30(2), 159 (1958); through Biol. Abstr. 33, 23368 (1961).

58. Giacosa, Morari. Gazz. chim. ital. 17, 362 (1887); through reference 60.

59. Giacosa, Soave. Gazz. chim. ital. 19, 303 (1889); through reference 60.

60. R. R. Paris, H. Moyse-Mignon. Ann. pharm. françaises 5, 410 (1947).

61. R. R. Paris, H. Moyse-Mignon. Ann. pharm. françaises 6, 409 (1948).

62. K. H. Palmer. Thesis, University of Paris (1956).

63. K. H. Palmer. Thesis, University of Paris, 63 (1956).

64. K. H. Palmer, R. R. Paris. Ann. pharm. françaises 13, 657 (1955).

65. K. H. Palmer. Thesis, University of Paris, 70 (1956).

66. K. H. Palmer. Thesis, University of Paris, 79 (1956).

67. R. R. Paris, H. Moyse-Mignon. Ann. pharm. françaises 9, 479 (1951).

68. G. V. Stuckert, Sartori. Rev., Univ. Nac. de Cordoba, Argentina, 19, 12 (1932); through reference 51.

69. V. Deulofeu, R. Labriola, Orias, Moisset de Espanés, Taquini. Science 102, 69 (1945).

70. Alkaloid bearing plants and their contained alkaloids. U. S. Dept. of Agriculture Technical Bulletin No. 1234.

71. S. C. Pakrashi, S. K. Roy, L. F. Johnson, T. George, C. Djerassi. Chem. and Ind. 1961, 464.

72. D. K. Chakravarti et al. Tetrahedron, 16, 224 (1961).

73. Ref. 62, page 91.